

**Notice of Allowability**

Application No.

09/751,299

Examiner

Chih-Min Kam

Applicant(s)

MADDEN ET AL.

Art Unit

1656

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 6/29/07.
2. ☒ The allowed claim(s) is/are 31,32,36,37,44,49,50,52-66 and 68-80.
3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some\* c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\* Certified copies not received: \_\_\_\_\_.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
- (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
- 1) ☐ hereto or 2) ☐ to Paper No./Mail Date \_\_\_\_\_.
- (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date \_\_\_\_\_.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

- |   |   |
|---|---|
| 1. <input type="checkbox"/> Notice of References Cited (PTO-892)  | 5. <input type="checkbox"/> Notice of Informal Patent Application                     |
| 2. <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)  | 6. <input type="checkbox"/> Interview Summary (PTO-413),<br>Paper No./Mail Date _____ |
| 3. <input checked="" type="checkbox"/> Information Disclosure Statements (PTO/SB/08),<br>Paper No./Mail Date <u>5/29/07</u> | 7. <input checked="" type="checkbox"/> Examiner's Amendment/Comment                   |
| 4. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit<br>of Biological Material                  | 8. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance  |
|   | 9. <input type="checkbox"/> Other _____   |

## DETAILED ACTION

### *Status of the Claims*

1. Claims 31, 32, 36, 37, 44, 49, 50, 52-66 and 68-80 are pending.

Applicants' amendment filed June 29, 2007 is acknowledged. Applicants' response has been fully considered. Claims 44, 50, 52, 53, 57, 59, 62, 68-70, 72-74 and 78 have been amended. Therefore, claims 31, 32, 36, 37, 44, 49, 50, 52-66 and 68-80 are examined.

### *Withdrawn Claim Rejections -- 35 USC § 112*

2. The previous rejection of claims 44, 50 and 68-80 under 35 U.S.C. 112, first paragraph, scope of enablement, is withdrawn in view of applicant's amendment to the claims, and applicant's response at pages 14-15 in the amendment filed June 29, 2007.
3. The previous rejection of claims 36, 37, 50, 52-65, 69 and 70 under 35 U.S.C. 112, second paragraph, is withdrawn in view of applicant's amendment to the claims, and applicant's response at page 15 in the amendment filed June 29, 2007.

### *Examiner's Amendment*

An **Examiner's Amendment** to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Gregory Einhorn on July 12, 2007.

#### **Examiner's Amendment to the Specification:**

Please replace the abstract filed July 22, 2003 with the following Abstract:

### ABSTRACT

The present invention provides methods for producing enantiomerically pure  $\alpha$ -substituted carboxylic acids such as  $\alpha$ -amino acids and  $\alpha$ -hydroxy acids, the method comprising combining an aldehyde or ketone with a cyanide and ammonia, an ammonium salt or an amine, in the presence of a nitrilase or a polypeptide having nitrilase activity which stereoselectively hydrolyzes the amino nitrile or cyanohydrin intermediate under conditions sufficient to produce the enantiomerically pure  $\alpha$ -substituted carboxylic acid such as  $\alpha$ -amino acids and  $\alpha$ -hydroxy acids.

**Examiner's Amendment to the Claims:**

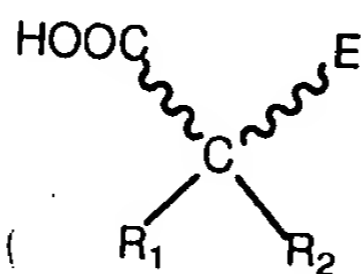
Claims 44, 49, 50, 57-60, 66, 68-70, 72-76, 78 and 79 have been amended as follows:

44. (Currently amended) A method for producing an alpha-substituted carboxylic acid, said method comprising

contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound, an ammonium salt or an amine under conditions to produce an amino nitrile or a cyanohydrin intermediate, and

hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a nitrilase, wherein the nitrilase hydrolyzes the intermediate in the presence of the reaction components to produce an alpha-substituted carboxylic acid and wherein the nitrilase has (i) an amino acid sequence having at least 95% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 95% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3, wherein the nucleic acid encodes a nitrilase enzyme,

wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

R<sub>1</sub> and R<sub>2</sub> are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R<sub>1</sub> and R<sub>2</sub> are linked to cooperate to form a functional cyclic moiety, and

E is -N(R<sub>x</sub>)<sub>2</sub> or -OH, wherein each R<sub>x</sub> is each independently -H or lower alkyl.

49. (Currently amended) A method for stereoselectively producing an alpha-substituted carboxylic acid, the method comprising

providing a composition comprising a nitrilase, wherein the nitrilase has an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or a enzymatically active fragment

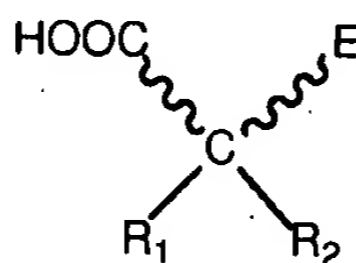
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thereof, wherein the fragment retains the enzymatic function of SEQ ID NO:2 or SEQ ID NO:4;  
and

contacting reaction components with the composition such that the nitrilase  
stereoselectively hydrolyzes the resulting amino nitrile or cyanohydrin intermediate in the  
presence of the reaction components to produce an alpha-substituted carboxylic acid,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing  
compound, and an ammonia-containing compound, ammonia salt, or amine,

wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

$\text{R}_1$  and  $\text{R}_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $\text{R}_1$  and  $\text{R}_2$  are linked to cooperate to form a functional cyclic moiety, and

$\text{E}$  is  $-\text{N}(\text{R}_x)_2$  or  $-\text{OH}$ , wherein each  $\text{R}_x$  is each independently -H or lower alkyl.

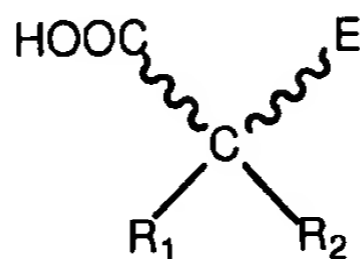
50. (Currently amended) A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising mixing reaction components to produce an amino nitrile or a cyanohydrin intermediate, and hydrolyzing stereoselectively the amino nitrile or cyanohydrin intermediate in the presence of the reaction components with a nitrilase,

wherein the reaction components are an aldehyde or ketone, a cyanide-containing compound, and an ammonia-containing compound, ammonia salt, or amine,

wherein the nitrilase has (i) an amino acid sequence having at least 95% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4 wherein the amino acid sequence retains the same biological activity as SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 95% sequence identity to an nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3 wherein the nucleic acid encodes a polypeptide having the same biological activity as SEQ ID NO:2 or SEQ ID NO:4,

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wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

$\text{R}_1$  and  $\text{R}_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $\text{R}_1$  and  $\text{R}_2$  are linked to cooperate to form a functional cyclic moiety, and

E is  $-\text{N}(\text{R}_x)_2$  or  $-\text{OH}$ , wherein each  $\text{R}_x$  is each independently -H or lower alkyl.

57. (Currently amended) The method of claim 31 or 32 or 66, wherein said  $\alpha$ -substituted carboxylic acid is an  $\alpha$ -hydroxy carboxylic acid.

58. (Currently amended) The method of claim 57, wherein said  $\alpha$ -hydroxy carboxylic acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

59. (Currently amended) The method of claim 31 or 66, wherein said cyanide-containing compound ~~comprises~~ is a metal cyanide or a gaseous cyanide.

60. (Currently amended) The method of claim 59, wherein said cyanide-containing compound ~~comprises~~ is an alkali cyanide.

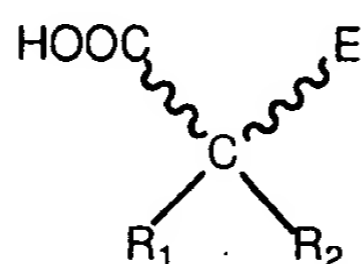
66. (Currently amended) A method for stereoselectively producing an alpha-substituted carboxylic acid, said method comprising

(a) providing a polypeptide having nitrilase activity, wherein the ~~nitrilase~~ polypeptide is encoded by a nucleic acid that hybridizes under stringent conditions to a sequence ~~as set forth in~~ consisting of SEQ ID NO: 1 or SEQ ID NO:3, and the stringent hybridization conditions comprise hybridization in a solution comprising ~~0.1-5M NaCl, 10% formamide, for 15 minutes at 72°C~~ 6 x SSC, 5 x Denhardt's reagent, 0.5% SDS, salmon sperm DNA and 50% formamide at 42 °C, and a wash step comprising a wash ~~in a buffer~~ comprising ~~150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na2EDTA,~~ 0.1 x SSC, 0.5% SDS, at between hybridization room temperature and 68 °C for 30 minutes;

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(b) contacting a composition comprising an aldehyde or ketone moiety with a cyanide-containing compound and an ammonia-containing compound, an ammonium salt or an amine, thereby producing an amino nitrile or cyanohydrin intermediate; and

(c) hydrolyzing stereoselectively the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity to produce an alpha-substituted carboxylic acid, wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

$R_1$  and  $R_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $R_1$  and  $R_2$  are linked to cooperate to form a functional cyclic moiety, and

E is  $-N(R_x)_2$  or  $-OH$ , wherein each  $R_x$  is each independently -H or lower alkyl.

68. (Currently amended) The method of claim 44, wherein the nitrilase has (i) an amino acid sequence having at least 97% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 95% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

69. (Currently amended) The method of claim 50, wherein the nitrilase has (i) an amino acid sequence having at least 97% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 97% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

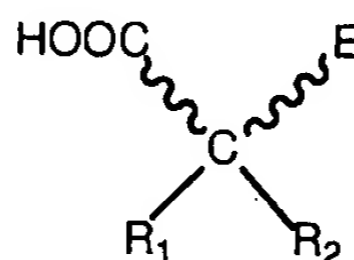
70. (Currently amended) The method of claim 69, wherein the nitrilase has (i) an amino acid sequence having at least 99% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 99% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

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72. (Currently amended) A method for producing an alpha-substituted carboxylic acid, said method comprising

- (a) providing a composition comprising an aldehyde or ketone, and a cyanide-containing compound and an ammonia-containing compound, an ammonium salt or an amine;
- (b) providing a polypeptide having nitrilase activity, wherein the polypeptide has an ~~amino acid sequence comprising~~ (i) an amino acid sequence having at least 95% sequence identity to a sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence encoded by a nucleic acid having at least 95% sequence identity to a nucleic acid sequence consisting of SEQ ID NO: 1 or SEQ ID NO:3;
- (c) contacting the composition with the cyanide-containing compound and the ammonia-containing compound, ammonium salt or amine under conditions wherein an amino nitrile or cyanohydrin intermediate is produced; and
- (d) hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity, thereby hydrolyzing the intermediate in the presence of the reaction components to produce an alpha-substituted carboxylic acid,

wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

$R_1$  and  $R_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $R_1$  and  $R_2$  are linked to cooperate to form a functional cyclic moiety, and

E is  $-N(R_x)_2$  or  $-OH$ , wherein each  $R_x$  is each independently -H or lower alkyl.

73. (Currently amended) The method of claim 72, wherein the polypeptide having nitrilase activity has (i) an amino acid sequence having at least 97% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 97% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

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74. (Currently amended) The method of claim 73, wherein the nitrilase having nitrilase activity has (i) an amino acid sequence having at least 99% sequence identity to an amino acid sequence consisting of SEQ ID NO:2 or SEQ ID NO:4, or (ii) an amino acid sequence is encoded by a nucleic acid having at least 99% sequence identity to a nucleic acid sequence consisting of SEQ ID NO:1 or SEQ ID NO:3.

75. (Currently amended) The method of claim 72, wherein the  $\alpha$ -substituted carboxylic acid is D-phenylalanine, D-phenylglycine, L-methylphenylglycine, L-tert-leucine, D-alanine, D-hydroxynorleucine, ~~R-pantolactone~~, 2-chloromandelic acid, (S)-mandelic acid, (R)-mandelic acid or (S)-cyclohexylmandelic acid.

76. (Currently amended) The method of claim 72, wherein the polypeptide having nitrilase activity hydrolyzes the amino nitrile or cyanohydrin intermediate in the presence of the reaction components to stereoselectively produce an alpha-substituted carboxylic acid.

78. (Currently amended) A method for producing an alpha-substituted carboxylic acid, said method comprising

(a) providing a polypeptide having nitrilase activity, wherein the polypeptide (i) is encoded by a nucleic acid that hybridizes under stringent conditions to a sequence ~~as set forth in~~ consisting of SEQ ID NO:1 or SEQ ID NO:3, and the stringent hybridization conditions comprise hybridization in a solution comprising ~~0.1-5M NaCl, 10% formamide, for 15 minutes at 72°C~~ 6 x SSC, 5 x Denhardt's reagent, 0.5% SDS, salmon sperm DNA and 50% formamide at 42 °C, and a wash step comprising a wash ~~in a buffer comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na<sub>2</sub>EDTA, 0.1 x SSC, 0.5% SDS, at between hybridization room temperature and 68 °C~~ for 30 minutes, or, (ii) has an amino acid sequence having at least 95 % sequence identity to a sequence ~~as set forth in~~ consisting of SEQ ID NO:2 or SEQ ID NO:4;

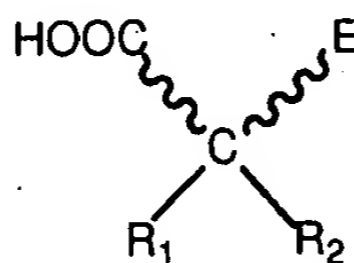
(b) providing a composition comprising an aldehyde or ketone moiety;

(c) contacting the composition with a cyanide-containing compound and an ammonia-containing compound, an ammonium salt or an amine, thereby producing an amino nitrile or cyanohydrin intermediate; and

(d) hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with the polypeptide having nitrilase activity, thereby hydrolyzing the intermediate in the presence of the reaction components to produce an alpha-substituted carboxylic acid,

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wherein said  $\alpha$ -substituted carboxylic acid has the following structure:



wherein:

$R_1$  and  $R_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $R_1$  and  $R_2$  are linked to cooperate to form a functional cyclic moiety, and

E is  $-N(R_x)_2$  or  $-OH$ , wherein each  $R_x$  is each independently -H or lower alkyl.

79. (Currently amended) The method of claim 78, wherein the polypeptide having nitrilase activity hydrolyzes the amino nitrile or cyanohydrin intermediate in the presence of the reaction components to stereoselectively produce an alpha-substituted carboxylic acid.

The following is an **Examiner's Statement of Reasons for Allowance**: The following references appear to be related to the claimed invention. Wakamoto *et al.* (U.S. Patent 5,587,303) teach the contact of  $\alpha$ -aminonitriles with microorganisms or enzyme extracts thereof having nitrile-hydrolyzing activity, and the contact produces the corresponding L-amino acids with enantiomeric purity; and Bhalla *et al.* (Applied Micro. Biotech 37, 184-190 (1992)) teach the reaction of various  $\alpha$ -amino nitriles with a nitrilase from *Rhodococcus* to produce their corresponding amino acids. However, the references do not teach or suggest a method of stereoselectively producing an alpha-substituted carboxylic acid by mixing reaction components of an aldehyde or a ketone with a cyanide-containing compound and an ammonia-containing compound or an amine to produce an amino nitrile or a cyanohydrin intermediate, and hydrolyzing stereoselectively the amino nitrile or cyanohydrin intermediate with a nitrilase consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or their functional fragments, or the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO:1 or 3. Therefore, the claims are allowable over the art of record.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Chih-Min Kam whose telephone number is (571) 272-0948. The examiner can normally be reached on 8.00-4:30, Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Bragdon can be reached at 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Chih-Min Kam, Ph. D.  
Primary Patent Examiner



CHIH-MIN KAM  
PRIMARY EXAMINER

CMK

July 13, 2007